

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

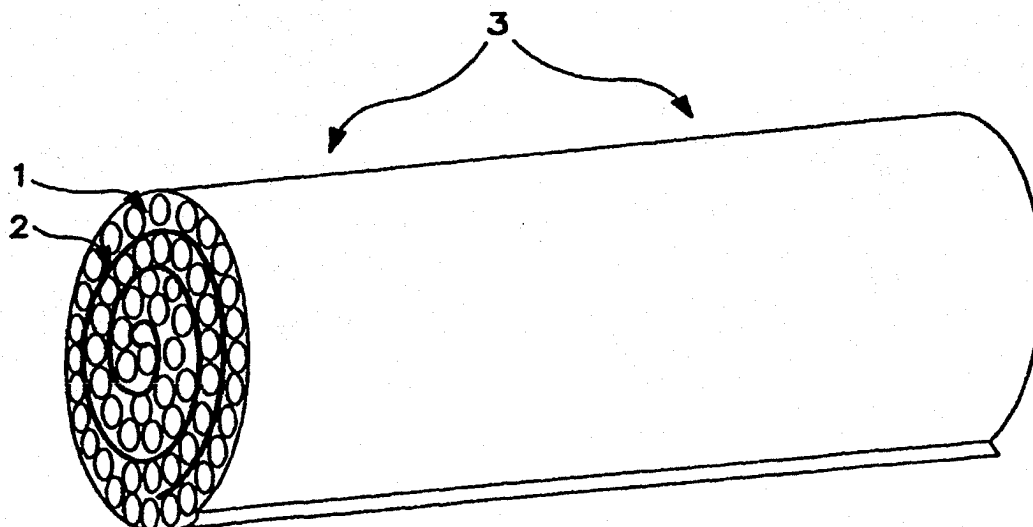


(2)

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> : B01D 63/02, 63/10, 69/10</p>	<p>A1</p>	<p>(11) International Publication Number: WO 99/07458 (43) International Publication Date: 18 February 1999 (18.02.99)</p>
<p>(21) International Application Number: PCT/US98/15476 (22) International Filing Date: 24 July 1998 (24.07.98) (30) Priority Data: 08/907,307 6 August 1997 (06.08.97) US (71) Applicant: GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US). (72) Inventor: VAN REIS, Robert, D.; 421 Hillcrest Way, Redwood City, CA 94062 (US). (74) Agents: CONLEY, Deirdre, L. et al.; Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report.</p>

(54) Title: HOLLOW FIBER CO-FLOW FILTRATION DEVICE



(57) Abstract

The present invention is directed to hollow fiber membrane filtration modules and processes useful for improved tangential flow filtration-mediated purification and separation of molecules from mixtures of molecules, wherein those molecules may be of biological interest. The hollow fiber membrane filtration modules of the present invention are such that the transmembrane pressure along the entire length of the hollow fibers is substantially constant, thereby providing enhanced filtration capabilities, especially for large-scale recombinant protein purification. The hollow fiber filtration modules of the present invention may be employed either with or without co-current recirculation of the filtrate.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

## HOLLOW FIBER CO-FLOW FILTRATION DEVICE

### FIELD OF THE INVENTION

The present invention relates to devices and processes useful for improved hollow fiber membrane filtration-mediated purification and separation of molecules from mixtures of molecules, wherein those molecules may be of biological interest. More specifically, the present invention provides a hollow fiber filtration device and process useful for controlling transmembrane pressure along the length of a hollow fiber membrane during filtration of complex macromolecular mixtures.

### BACKGROUND OF THE INVENTION

Several methods are currently available for separating and purifying molecules of biological interest, such as proteins, from complex mixtures thereof. One of the most well known processes for separating and purifying molecules of interest is membrane filtration; a process which separates dissolved and suspended solutes in solutions based upon their size. In its simplest form, membrane filtration involves the passage of a solution containing the molecule of interest through a filtration membrane having pores of a defined size. Solute larger than the membrane pore size are retained, while solutes smaller than the pore size pass through the membrane with the solvent, thereby effectively separating the larger solutes from the smaller solutes.

Such membrane filtration processes generally fall within the categories of reverse osmosis, microfiltration and ultrafiltration depending upon the pore size of the membrane employed. Conventionally, reverse osmosis employs filtration membranes which are capable of retaining salts and other low molecular weight solutes whereas microfiltration, or microporous filtration, employs membranes in the 0.1 to 10 micron pore size range, typically used to retain colloids and microorganisms. Ultrafiltration, on the other hand, employs filtration membranes rated for retaining solutes between approximately 1 and 1000 kDa in molecular weight and has proven useful for processing up to thousands of liters of solution per hour, specifically for protein concentration and the removal of low molecular weight contaminants from large-scale recombinant protein preparations.

While the ultrafiltration process has proven useful for a variety of applications, limitations do exist on the degree of protein purification achievable by the process. These limitations are due mainly to the well known phenomena of concentration polarization, fouling and wide membrane pore size distribution. See, e.g., Porter, ed., *Handbook of Industrial Membrane Technology* (Noyes Publications, Park Ridge, N.J., 1990), pp. 164-173. As a result of these limitations, difficulties have been encountered in the large scale resolution of macromolecular mixtures. See Michaels, "Fifteen Years of Ultrafiltration: Problems and Future Promises of an Adolescent Technology", in Anthony R. Cooper, ed., *Ultrafiltration Membranes and Applications, Polymer Science and Technology*, 13 (Plenum Press, N.Y., 1979), pp. 1-16, at p. 9. Thus, the efficiency of ultrafiltration in large scale complex macromolecular mixture separations has proven to be less than desirable.

In an attempt to overcome the above described limitations of the ultrafiltration process, however, modifications of the process have been developed. One important such modification is the development of the tangential flow filtration (TFF) process. In the TFF process, a solution is passed over a filtration membrane at a high velocity and tangential to the plane of the membrane surface so as to enhance transport of membrane-retained solutes away from the membrane surface and back into the retentate. One major problem in tangential flow filtration, however, is the creation of a differential transmembrane pressure (TMP)

gradient along the length of the filtration membrane. Generally, the TMP at the portion of the filtration membrane nearest the feed inlet is very high and it progressively decreases along the length of the filtration membrane as the fluid moves away from the feed inlet toward the retentate outlet end of the filtration device, where the TMP may be very low. As higher feed-side velocities are employed to combat concentration polarization and fouling, the differential TMP gradient over the length of the membrane increases.

This differential TMP gradient causes a number of drawbacks. For example, one drawback of a differential TMP gradient in the filtering passage is that the operational conditions of the filter vary along the entire length thereof. As such, the TMP over a very large percentage of the filter surface will vary considerably from that which provides for optimal filtration efficiency.

Efforts have been made, however, to control the TMP gradient over the length of the filtration membrane so as to provide for a substantially constant TMP across the entire filter surface. For example, U.S. Patent No. 5,256,294, issued on October 26, 1993 to van Reis, describes a high performance tangential flow filtration (HPTFF) process which is performed under certain conditions of flux, where the flux ranges from 5% to up to 100% of the transition point flux, and TMP, where the TMP is held at a range in the pressure dependent region of the flux versus TMP curve, namely, at a range that is no greater than the TMP value at the transition point. In this process, the filtrate is recirculated in a co-current mode. By this mechanism, when the filtrate flow on the filtrate side of the membrane is such that the pressure drop on the filtrate side of the membrane is similar to that on the retentate side of the membrane, a substantially constant TMP is maintained over the entire length of the membrane, thereby allowing one to obtain an optimal TMP over the entire length of the membrane. Recirculation of the filtrate in parallel to the feed flow is also disclosed in U.S. Patent No. 4,105,547 by Sandblom as providing a means for obtaining a substantially constant TMP across the entire length of the filtration membrane.

However, while co-current filtrate recirculation has proven to be useful for controlling the TMP gradient in flat sheet filtration membrane systems, it has been less than effective in systems employing hollow fiber filtration membranes. This is because the co-current filtrate recirculation rate has to be very high, often higher than the feed rate, in order to accomplish an equal TMP across the length of the hollow fibers because of the very low resistance on the shell side of a hollow fiber cartridge. As such, other methods have been employed in hollow fiber filtration systems in attempts to maintain substantially constant TMPs over the entire length of the hollow fiber. For example, U.S. Patent No. 5,525,144 by Gollan discloses a hollow fiber filtration cartridge that has filtrate flow restrictions embedded into the intracartridge potting material which serve as obstacles to filtrate flow, thereby controlling the pressure drop along the filtrate side of the hollow fiber and, in turn, reducing variations in TMP along the length of the fiber. However, because the filtrate flow restrictions are spaced in such a way as to create intracartridge "subcompartments", the pressure drop on the filtrate side of the membrane is not linear, thereby making it difficult to control TMP variance in an exact manner. Moreover, such hollow fiber cartridges are difficult and expensive to manufacture. As such, there is a need for hollow fiber filtration devices which are relatively easy to manufacture and which provide for a linear pressure drop on the filtrate side of the membrane.

Therefore, it is an object of the present invention to provide novel filtration devices and processes for improved filtration in hollow fiber filtration systems.

Another object of the present invention is to provide a hollow fiber filtration device which allows one to maintain a relatively constant TMP over the entire length of the hollow fiber membrane when co-current recirculation of the filtrate is employed.

Another object of the present invention is to provide a hollow fiber filtration device which allows one to conduct efficient hollow fiber membrane filtration even when co-current recirculation of the filtrate is not employed.

Still another object of the present invention is to provide a hollow fiber filtration device which provides a substantially linear pressure drop profile on the filtrate side of the fiber, thereby providing a means for maintaining a substantially constant TMP over the entire length of the hollow fiber membrane.

Yet another object of the present invention is to provide a hollow fiber filtration device which is inexpensive and easy to manufacture and utilize.

Numerous other features, objects and advantages will become apparent to those skilled in the art from the following description of the present invention.

#### SUMMARY OF THE INVENTION

In accordance with the present invention, novel filtration devices and processes are provided which are useful for improved tangential flow-mediated purification and separation of molecules from mixtures of molecules, wherein those molecules may be of biological interest. More specifically, the present invention provides a hollow fiber membrane filtration module useful for separating species of interest from a mixture, wherein said filtration module comprises (a) a filter cartridge shell and (b) a hollow fiber filter element contained within the filter cartridge shell. As employed in the presently described filtration module, the hollow fiber filter element comprises a mat of hollow fibers which is physically layered onto the surface of a screen, wherein the hollow fibers of the filter element are configured into a bundle and the screen occupies at least a portion of the void volume between the hollow fibers. By occupying at least a portion of the void volume between the hollow fibers, the screen functions to control the pressure drop on the filtrate side of the hollow fiber membrane, thereby allowing one to obtain a substantially constant transmembrane pressure along the entire length of the hollow fibers in the filtration module. In place of the screen, however, in another embodiment of the present invention, a portion of the void volume between the hollow fibers may be occupied by a material which functions to weave the substantially parallel hollow fibers into a mat. This material may also provide a significant restriction to flow on the filtrate side of the membrane, thereby allowing one to maintain a substantially constant TMP across the entire length of the hollow fibers in the filtration module. The hollow fiber filtration modules of the present invention may be employed either with or without co-current recirculation of the filtrate on the filtrate side of the hollow fibers.

In certain preferred embodiments, the hollow fiber mat is a monolayer mat wherein the hollow fibers of the mat may optionally be interwoven with a material that provide restriction to flow on the filtrate side of the fibers. In other embodiments, the hollow fibers employed may be used for microfiltration or ultrafiltration, in the latter where the fibers are sufficient for retaining solutes from about 1 to 1000 kDa in size. The filtration module of the present invention is useful for separating a variety of different species from mixtures, wherein in preferred embodiments, those species are cells, proteins, viruses, and the like.

Another aspect of the present invention is directed to a method of making a hollow fiber filtration module useful for separating species of interest from a mixture, wherein the method comprises:

- (a) providing a mat of hollow fibers;
- (b) physically layering the mat of hollow fibers onto a screen to provide a hollow fiber mat/screen complex;
- (c) configuring the hollow fiber mat/screen complex into a form wherein the hollow fibers are in a bundle and the screen occupies at least a portion of the void volume between the hollow fibers, thereby providing a hollow fiber filter element; and
- (d) inserting the hollow fiber filter element into a filter cartridge shell, wherein the hollow fiber filter element is positioned within the filter cartridge shell so as to allow filtration of a fluid through the hollow fiber filter element.

In a preferred embodiment, the step of configuring the hollow fiber mat/screen complex comprises rolling that complex into a substantially cylindrical shape. The hollow fiber mat may optionally be permanently attached to the screen.

Yet another aspect of the present invention is directed to a method of separating a species of interest from at least one component of a mixture, wherein the method comprises filtering a fluid containing the species of interest through one of the hollow fiber membrane filtration modules described above, wherein at least a portion of the pore sizes of the hollow fibers present in the filtration module are sufficiently small so as to not allow passage of the species of interest yet are sufficiently large to allow passage of one or more other components. In preferred embodiments, the species of interest is a recombinantly produced protein, a cell or a cellular component. Often, the pore sizes of the hollow fibers employed are rated for retaining solutes in the range of about 1 to 1000 kDa in size.

Other aspects of the present invention will become evident to those of ordinary skill in the art upon a reading of the present specification.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows one embodiment of the present invention wherein a monolayer hollow fiber filter mat 1 is physically layered onto a screen 2 such that when the hollow fibers are configured into a bundle to form a hollow fiber filter element, the screen 2 will occupy at least a portion of the void volume between the hollow fibers in the bundle.

Figure 2 shows a cross-sectional view of a monolayer hollow fiber filter mat 1 which is physically layered onto a screen 2.

Figure 3 shows a hollow fiber filter element 3 which has been rolled into a substantially cylindrical shape wherein the hollow fibers of the hollow fiber mat 1 and the screen 2 upon which the hollow fiber mat is physically layered are arranged in an internal substantially spiral structure.

#### **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

##### **A. Definitions**

As used herein, a "hollow fiber membrane filtration module" means a filtration apparatus which is useful for separating species of interest from a mixture and which comprises both (1) a filter cartridge shell and (2) a hollow fiber filter element contained within that cartridge shell. As used herein, the expression

"hollow fiber filter element" and grammatical equivalents thereof refer to the portion of the filtration module which is inserted into the filter cartridge shell and which comprises a mat of hollow fibers which are physically layered onto the surface of a support screen. In other embodiments, however, the hollow fibers of the hollow fiber filter element may be configured substantially parallel and interwoven with a material which occupies at least a portion of the void volume between the fibers, thereby providing a restriction to flow on the filtrate side of the fibers. In the latter embodiment, the hollow fiber filter element may optionally comprise a screen.

As described in detail below, once the mat of hollow fibers is physically layered onto the surface of the screen, it is conditioned into a shape where the hollow fibers are configured into a bundle and where the screen takes up or occupies at least a portion of the void volume between the hollow fibers. Preferably, the hollow fiber mat/screen complex is rolled into a substantially cylindrical shape which has an internal structure wherein the hollow fibers take a multi-layered spiral shape wherein each layer of hollow fibers in the spiral is separated by a layer of the screen. Further detail as to the components of the hollow fiber filter element will be provided below.

As used herein, the expression "filter cartridge shell" means the component of the complete filtration module which serves to encompass and support the hollow fiber filtration element. The cartridge shell may take many different forms, preferably cylindrical, and sizes, depending upon the form and size of the hollow fiber filter element. Many cartridge shells are well known in the art and are commercially available.

As used herein, the expression "physically layered" when used in regard to the hollow fiber mat and screen means that the hollow fiber mat is placed directly upon a screen to provide a means for increasing the resistance to filtrate flow between the hollow fibers when present in a hollow fiber filter element, thereby significantly reducing the pumping capacity required to maintain a substantially constant TMP along the length of the hollow fibers. The hollow fiber mat may then be physically and/or irreversibly attached to the screen by means well known to those of ordinary skill in the art, although such is not critical to the present invention.

Generally, the hollow fiber filter element is maintained within the cartridge shell of the filtration module by conventional potting technology which is well known in the art. The filtration module will generally have feed inlet and retentate and filtrate outlets through which a fluid may be introduced into the module prior to filtration and recovered from the module after filtration, respectively. Various different filtration module designs are well known in the art and may be employed in the filtration module of the present invention.

As used herein, the term "species" generally means a particle(s) or molecule(s) that is to be separated from a solution or suspension in a fluid, e.g., a liquid. The species are separated from the fluid and, in most instances, from other particles or molecules in the fluid. The size of the species of interest to be separated will determine the pore size of the hollow fibers to be employed. Preferably, the species are biological entities of natural biological or biochemical origin or produced by biological or biochemical processes. Examples of preferred species include, without being limiting of the invention, mammalian cells and microorganisms such as bacteria, fungi and yeast, as well as species of suitable size for ultrafiltration, for example, polypeptides, proteins, either naturally or recombinantly produced, cellular components, DNA, colloids, mycoplasma, endotoxins, viruses, bacteria, carbohydrates, and other biological molecules or interest, whether glycosylated or not. In one embodiment, the species of interest are biological molecules having a molecular weight of at least about 1000 daltons and most preferably are polypeptides or proteins. In yet another

embodiment, the herein described hollow fiber filtration module employs fibers having a pore size of from 0.1 to 1 micron or larger for use in microfiltration.

As used herein, a "mixture" of species refers to a fluid solution or suspension which contains two or more different species. The types of species present in any mixture may differ greatly, wherein a mixture may contain, for example, various different polypeptides, buffers, salts, cells and cell components, and the like. Mixtures may be complex, wherein a large number of different species are represented, or relatively simple, where only a small number of different species are represented.

As used herein, the expression "transmembrane pressure" or "TMP" refers to the pressure differential gradient that is applied along the length of a hollow fiber filtration membrane to cause fluid and filterable solutes to flow through the pores of the filter.

As used herein, the expression "substantially constant" as applied to TMP refers to a situation where the TMP at the feed inlet is no more than about 2-fold greater than the TMP at the retentate outlet. It is to be understood, however, that for various applications the ratio of TMP at the feed inlet versus TMP at the retentate outlet of the hollow fiber filtration module may vary considerably, but this ratio is usually less than about 1.8, preferably less than about 1.5 and more preferably less than about 1.3.

As used herein, the expression "same pore size" as applied to the hollow fiber filtration membranes refers to membranes which are rated or sold as having the same pore size, even though the actual pore sizes of the fibers may vary somewhat.

#### **B. Modes for Carrying Out the Invention**

In its broadest aspect, the hollow fiber filtration module of the present invention is useful for separating species of interest from a mixture of different species, wherein the filtration module comprises a filter cartridge shell and a hollow fiber filter element contained within the filter cartridge shell. In one embodiment, the hollow fiber filter element which is inserted into the filter cartridge shell comprises at least two components, i.e., (1) a mat of hollow fibers which is physically layered onto the surface of (2) a screen. The presence of the screen serves to occupy at least a portion of the void volume between the hollow fibers, thereby providing resistance to filtrate flow during filtration, thereby providing a substantially constant TMP across the entire length of the hollow fibers at a lower co-current flow rate.

In another embodiment, the screen as described above may optionally be replaced with a material which functions to weave the hollow fibers together and hold them in a substantially parallel configuration. Because the material which weaves the fibers together (i.e., the interweaving material) functions to occupy at least a portion of the void volume between the hollow fibers, this material may also provide a significant restriction to flow on the filtrate side of the fibers, thereby allowing one to maintain a substantially constant TMP across the entire length of the filtration module. Materials which can serve to weave the hollow fibers together are well known in the art and will be chosen such that they do not adversely effect the species of interest to be filtered from a solution. Interweaving materials generally occupy at least about 5% of the void volume between the hollow fibers, usually from about 5% to about 99% of the void volume between the fibers, preferably from about 15% to about 99% of the void volume and more preferably from about 50% to about 99% of the void volume. The mat of interwoven hollow fibers obtained is referred to herein as the "interwoven hollow fiber mat".



As to the hollow fibers which are incorporated into the filtration module of the present invention, various types of hollow fibers may be employed. For example, hollow fibers which find use in the present invention include hollow fibers formed from various materials based on cellulose, polyolefins, polyvinyl alcohol, PMMA, polysulfones, and the like. Other hollow fibers which find use herein include hollow fibers which are formed from materials such as polyethylene and polypropylene, such fibers being capable of being easily woven into fabrics. No particular limitation is placed upon the pore size of the hollow fibers employed, nor the porosity, fiber wall thickness and outer diameter of the fiber used, so long as they serve as a filtering membrane for the application desired. For the purification of a recombinantly produced protein from a complex mixture, generally pore sizes which are rated for retaining solutes between approximately 1 and 1000 kDa in molecular weight are preferred. Pore sizes of from about 0.1 to 10 microns are generally employed for the purification of biomolecules from cellular debris and intact cells. The hollow fibers employed herein may be prepared using methods which are well known to those of ordinary skill in the art and/or are commercially available.

The hollow fibers employed in the present invention are typically formed into a mat, which is preferably a monolayer mat, however, multiple layer hollow fiber mats may also find use herein. The fibers of a hollow fiber filter element are typically configured in a substantially parallel fashion as is well known and routinely employed in the art. The hollow fibers of a mat may optionally be woven together by a material which is placed substantially perpendicularly to the length of the fibers to form an interwoven hollow fiber mat as described above. Techniques for preparing interwoven hollow fiber mats having widely-spaced polymeric threads interwoven perpendicularly to the hollow fibers are well known to those of ordinary skill in the art. The hollow fiber mat may take virtually any shape and/or size depending upon the desired application, however square or rectangular shapes are generally preferred.

Once prepared, the mat of hollow fibers as described above may then be physically layered onto a surface of a screen, wherein the screen functions to provide a means for increasing the resistance to filtrate flow between the hollow fibers when present in a hollow fiber filter element, thereby significantly reducing the pumping capacity required to maintain a substantially constant TMP along the length of the hollow fibers. The screen is generally comprised of a perforated or fenestrated material such as a porous mesh or other screen-like material and may comprise any suitable material which provides for increased resistance to filtrate flow between the hollow fibers and which does not adversely react with the fluid to be filtered or any component of interest contained therein. The precise composition employed to produce the screen, as well as other parameters considered in the preparation of an operational hollow fiber filtration element, are well known and may be readily selected by those of ordinary skill in the art for particular end-uses. However, non-limiting examples of screen components which find use in the present invention include plastics, synthetic polymeric materials, metals including, for example, aluminum or stainless steel, and the like. Preferably, the screen takes a size and shape that is similar to that of the hollow fiber mat which is physically layered thereon, however, such is not critical to the invention and the size and shape of the screen may vary greatly depending upon the desired application. One embodiment of a hollow fiber mat 1 which is physically layered onto the surface of a screen 2 is shown in Fig. 1. Fig. 2 shows a cross-sectional view of a hollow fiber mat 1 which is physically layered onto the surface of a screen 2.

Once the mat of hollow fibers is physically layered onto the screen and optionally attached thereto as described above or once the interwoven hollow fiber mat is obtained, a hollow fiber filter element is prepared by conditioning the hollow fiber mat/screen complex or interwoven mat into a form where the hollow fibers are configured into a bundle and the screen or interweaving material occupies at least a portion of the void volume between the hollow fibers. By "bundle" is meant that the fibers of the mat are brought into close physical proximity. Preferably, the hollow fiber mat/screen complex or the interwoven hollow fiber mat is rolled into a substantially cylindrical shape which has an internal substantially spiral structure. Therefore, in one embodiment, internal to the hollow fiber filter element are hollow fibers arranged in a substantially spiral shape wherein the hollow fibers may be separated by a layer of screen or interweaving material. One embodiment of a hollow fiber/screen complex which has been configured into a substantially spiral shape is shown in Fig. 3.

By "substantially spiral" is meant that internal to the filter element, there are multiple substantially circular shaped layers of hollow fibers which begin from a central internal point and extend outwardly in a circular fashion. Therefore, unlike in conventional hollow fiber bundles, much of the void volume between the hollow fibers is replaced in the presently described element with a porous screen-like material or interweaving material which provides significant resistance to filtrate flow therethrough, thereby increasing the resistance and, in turn, significantly reducing the pumping capacity required to maintain a substantially constant TMP along the length of the hollow fibers. The hollow fiber mat/screen complex or interwoven hollow fiber mat may be rolled into a substantially cylindrical shape as tightly or as loosely as desired, thereby controlling the amount of void volume present in the hollow fiber filter element.

Once prepared, the substantially spiral shaped hollow fiber filter elements of the present invention may further be enclosed within a cartridge shell or housing unit such that a complete hollow fiber filtration module is formed. Housing unit or cartridge shell configurations, materials therefor, and method for their preparation, are well known to, and appreciated by, those of ordinary skill in the art. Generally, housing units and cartridge shells are prepared from inert materials so as to ensure that there is no adverse interaction between the housing unit or cartridge shell and the fluid to be filtered or any component of interest contained therein. The spiral shaped hollow fiber filter elements described above may be prepared and inserted into a hollow fiber cartridge shell and potted in place using conventional methodology, thereby providing a complete hollow fiber filtration cartridge or module.

The precise housing unit or cartridge shell composition employed and size parameters of the housing unit, as well as other parameters considered in the preparation of an operational hollow fiber filtration module, are well known and may be readily selected by those of ordinary skill in the art for particular end-uses.

The hollow fiber filtration modules of the present invention and processes for their use as described herein may be employed in a variety of applications, including, but not limited to, the separation and purification of one or more proteins from a complex mixture of different proteins, especially during large scale recombinant protein production. The filtration modules and processes of the present invention are applicable for separation and purification of a wide range of biological molecules, e.g., proteinaceous products of fermentation of natural or genetically engineered microorganisms, polypeptides, antibodies, cellular secretions, colloids, mycoplasma, endotoxins, viruses, bacteria, amino acids, DNA, RNA, carbohydrates, and the like.

The present invention also finds use for separating cellular debris from whole cells. For example, in continuous perfusion cultures, it would be desirable to not only exchange media components and remove products, but also to remove cellular debris that would otherwise accumulate during the run. In such a case, the hollow fiber pore size employed would be close to that of intact cells, thereby allowing cellular debris to flow into the filtrate while whole cells are retained in the retentate.

The presently described devices and processes may also be employed for separating inorganic contaminants from various mixtures containing organic and/or inorganic molecules. For example, proteins present in a complex mixture may be effectively separated and purified away from salts, buffers, and the like, which are commonly found in crude protein preparations. Components found in mixtures may also be effectively concentrated using the presently claimed invention.

Filtration as described above may be performed by passing an appropriate fluid of interest through the hollow fiber filtration element or complete filter module prepared as described above. Methods and techniques for doing so are well known in the art.

The present invention has a number of advantages over the existing prior art. First, by reducing or eliminating the requirement for filtrate recirculation to maintain a substantially constant TMP over the entire length of the filter element, the filtration system is more economical and less complex than other known filtrations systems. The use of a porous screen or an interweaving material in the hollow fiber filter element controls the pressure drop on the filtrate side of the membrane, thereby allowing one to maintain a substantially constant TMP over the length of the filtration cartridge. The filtration device of the present invention is also economical and easy to prepare.

The foregoing description details specific methods which can be employed to practice the present invention. Having detailed such specific methods, those skilled in the art will well enough know how to devise alternative reliable devices and methods at arriving at the same information in using the fruits of the present invention. Thus, however detailed the foregoing may appear in text, it should not be construed as limiting the overall scope thereof; rather, the ambit of the present invention is to be determined only by the lawful construction of the appended claims. All documents cited herein are expressly incorporated by reference.

**WHAT IS CLAIMED IS:**

1. A hollow fiber membrane filtration module useful for separating species of interest from a mixture, said filtration module comprising:
  - (a) a filter cartridge shell; and
  - (b) a hollow fiber filter element contained within said filter cartridge shell, said hollow fiber filter element comprising a mat of hollow fibers physically layered onto the surface of a screen, wherein the hollow fibers of said filter element are configured into a bundle and said screen occupies at least a portion of the void volume between said hollow fibers, thereby restricting the flow of filtrate between said hollow fibers.
2. The hollow fiber filtration module according to Claim 1, wherein said filtration module exhibits a substantially constant transmembrane pressure along the entire length of the hollow fibers in said module.
3. The hollow fiber filtration module according to Claim 1, wherein said mat of hollow fibers is a monolayer mat of hollow fibers.
4. The hollow fiber filtration module according to Claim 1, wherein said hollow fibers have a pore size of from about 1 to 1000 kDa.
5. The hollow fiber filtration module according to Claim 1, wherein said species of interest are selected from the group consisting of cells, proteins and viruses.
6. The hollow fiber filtration module according to Claim 5, wherein said cells are mammalian cells.
7. The hollow fiber filtration module according to Claim 5, wherein said cells are *E. coli* cells.
8. The hollow fiber filtration module according to Claim 1, wherein said species of interest is a component of an *E. coli* lysate.
9. The hollow fiber filtration module according to Claim 5, wherein said proteins are recombinantly expressed proteins.
10. The hollow fiber filtration module according to Claim 1, wherein said hollow fiber filter element has a substantially cylindrical shape.
11. A hollow fiber membrane filtration module useful for separating species of interest from a mixture, said filtration module comprising:
  - (a) a filter cartridge shell; and
  - (b) a mat of hollow fibers contained within said filter cartridge shell, wherein in said mat said hollow fibers are configured substantially in parallel and are interwoven together with a material which occupies at least about 5% of the void volume between said hollow fibers, thereby restricting the flow of filtrate between said hollow fibers.
12. The hollow fiber filtration module according to Claim 11, wherein said material occupies from about 15% to 99% of the void volume between said hollow fibers.

13. The hollow fiber filtration module according to Claim 11, wherein said filtration module exhibits a substantially constant transmembrane pressure along the entire length of the hollow fibers in said module.
14. The hollow fiber filtration module according to Claim 11, wherein said hollow fibers  
5 have a pore size of from about 1 to 1000 kDa.
15. The hollow fiber filtration module according to Claim 11, wherein said species of interest are selected from the group consisting of cells, proteins and viruses.
16. A method of making a hollow fiber filtration module useful for separating species of interest from a mixture, said method comprising:
- 10 (a) providing a mat of hollow fibers;
- (b) physically layering said mat of hollow fibers onto a screen to provide a hollow fiber mat/screen complex;
- (c) configuring said hollow fiber mat/screen complex into a form wherein said hollow fibers are in a bundle and said screen occupies at least a portion of the void volume  
15 between said hollow fibers, thereby providing a hollow fiber filter element; and
- (d) inserting said hollow fiber filter element into a filter cartridge shell, wherein said hollow fiber filter element is positioned within said filter cartridge shell so as to allow filtration of a fluid through said hollow fiber filter element.
17. The method according to Claim 16, wherein the step of configuring comprises rolling  
20 said hollow fiber mat/screen complex into a substantially cylindrical shape.
18. The method according to Claim 16, wherein said mat of hollow fibers is a monolayer mat.
19. The method according to Claim 16, wherein the step of physically layering comprises permanently adhering said mat of hollow fibers to said screen.
20. A method of separating a species of interest from at least one component of a mixture, said method comprising filtering a fluid containing said species of interest through the hollow fiber membrane filtration module of Claim 1 or 11, wherein at least a portion of the pore sizes of the hollow fibers present in said module are sufficiently small so as to not allow passage of said species of interest yet are sufficiently large to allow passage of said at least one component.
21. The method according to Claim 20, wherein said species of interest is a recombinantly  
30 expressed protein.
22. The method according to Claim 20, wherein said species of interest is a cell or a cell component.
23. The method according to Claim 20, wherein said at least a portion of the pore sizes  
35 are in the range of about 1 to 1000 kDa.
24. The method according to Claim 20 which further comprises the step of co-currently recirculating the filtrate on the filtrate side of said hollow fibers.

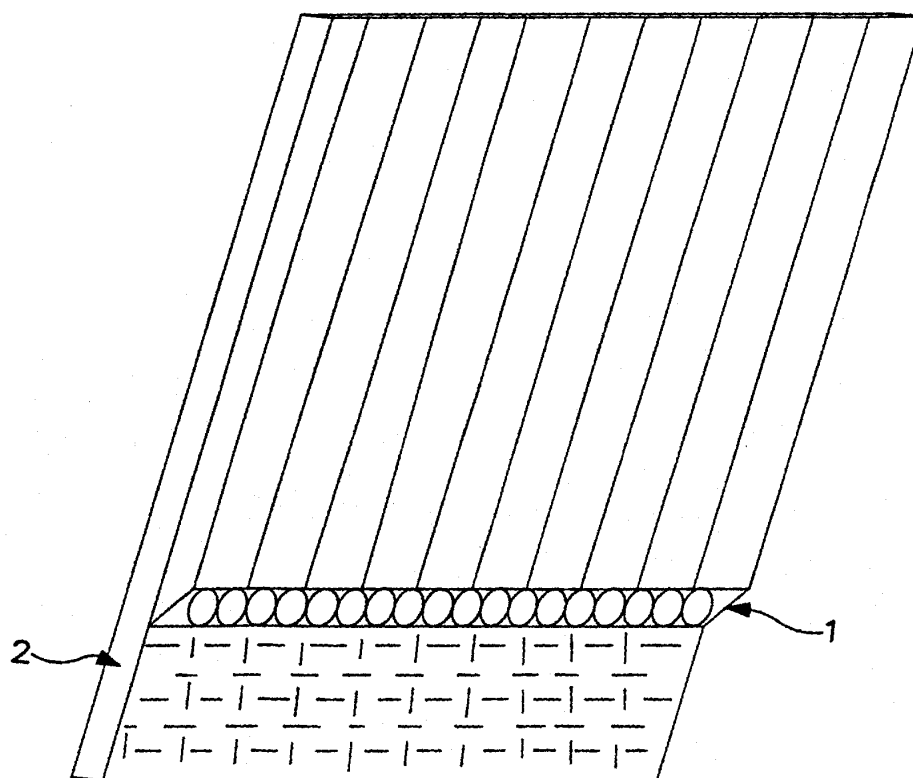


FIG. 1

2 / 2

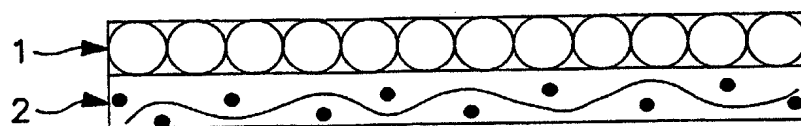


FIG. 2

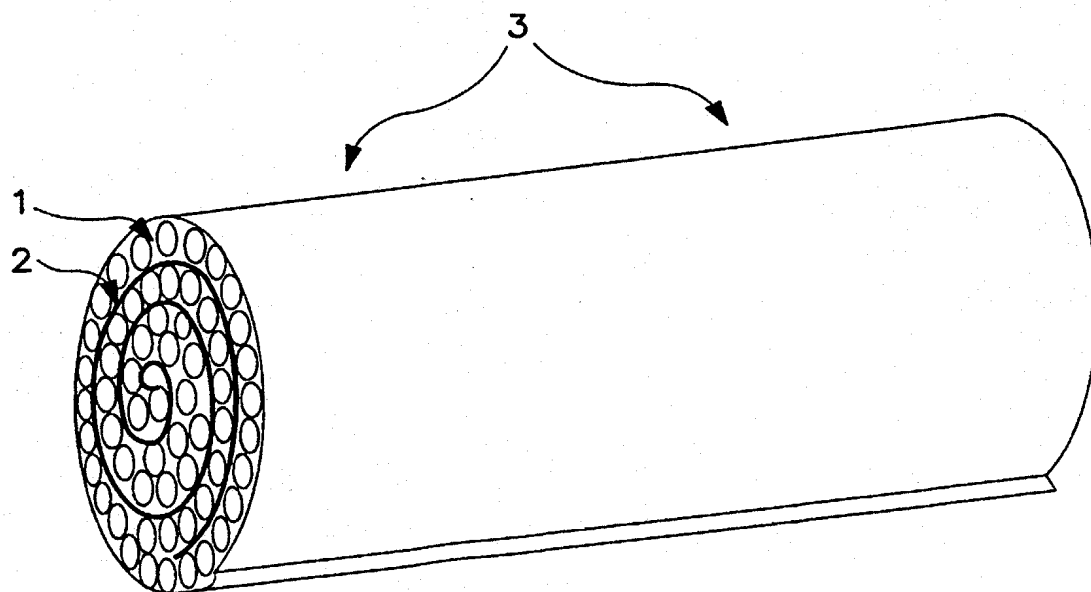


FIG. 3

# INTERNATIONAL SEARCH REPORT

Intern. Appl. No.  
PCT/US 98/15476

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 B01D63/02 B01D63/10 B01D69/10

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 9413 Derwent Publications Ltd., London, GB; AN 94-106079 XP002078769 & JP 06 055038 A (MITSUBISHI RAYON CO LTD) , 1 March 1994 see abstract	1, 3, 10, 16-19
X	PATENT ABSTRACTS OF JAPAN vol. 96, no. 011, 29 November 1996 & JP 08 187086 A (ASAHI CHEM IND CO LTD), 23 July 1996 see abstract	4, 5, 20, 23
	--- -/-	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 September 1998

Date of mailing of the international search report

07/10/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Cordero Alvarez, M



# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/15476

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 13, no. 238, 5 June 1989 & JP 01 051075 A (ASAHI CHEM IND CO LTD), 27 February 1989 see abstract ---	4,5,20, 23
A	PATENT ABSTRACTS OF JAPAN vol. 96, no. 5, 31 May 1996 & JP 08 023976 A (ASAHI CHEM IND CO LTD), 30 January 1996 see abstract -----	4-9,20, 23